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Review

Herpesviral Fc γ receptors: culprits attenuating antiviral IgG?

Matthias Budt, Henrike Reinhard, Arndt Bigl, Hartmut Hengel*

Robert Koch-Institute, Division of Viral Infections, Nordufer 20, 13353 Berlin, Germany

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Abstract

Production of IgG in response to virus infection is central to antiviral immune effector functions and a hallmark of B cell memory. Antiviral antibodies (Abs) recognising viral glycoproteins or protein antigen displayed on the surface of virions or virus-infected cells are crucial in rendering the virus noninfectious and in eliminating viruses or infected cells, either acting alone or in conjunction with complement. In many instances, passive transfer of Abs is sufficient to protect from viral infection. Herpesviruses (HV) are equipped with a large array of immunomodulatory functions which increase the efficiency of infection by dampening the antiviral immunity. Members of the α - and β -subfamily of the Herpesviridae are distinct in encoding transmembrane glycoproteins which selectively bind IgG via its Fc domain. The Fc-binding proteins constitute viral Fc γ receptors (vFc γ Rs) which are expressed on the cell surface of infected cells. Moreover, vFc γ Rs are abundantly incorporated into the envelope of virions. Despite their molecular and structural heterogeneity, the vFc γ Rs generally interfere with IgG-mediated effector functions like antibody (Ab)-dependent cellular cytotoxicity, complement activation and neutralisation of infectivity of virions. vFc γ Rs may thus contribute to the limited therapeutic potency of antiherpesviral IgG in clinical settings. A detailed molecular understanding of vFc γ Rs opens up the possibility to design recombinant IgG molecules resisting vFc γ Rs. Engineering IgG with a better antiviral efficiency represents a new therapeutic option against herpesviral diseases.

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1. Introduction: herpesvirus endurance between immune control and immune evasion

Herpesviruses (HV) which are subdivided into three subfamilies (α , β and γ) possess large double-stranded DNA genomes encoding approximately 100–200 genes. HV genomes remain latent in their natural host and can initiate a productive infection from latently infected cells resulting in virus shedding and transmission of virus progeny to a new host [1,2].

During the long history of coevolution and cospeciation with their hosts, HV adopted multiple mechanisms to deal with immune functions that permit establishing a life-long infection with efficient replication and transmission, even in the presence of repeatedly boosted antiviral immune responses [3,4]. Typically, the herpesviral evasion strategy prominently targets those immune responses that attack and contain the virus with the highest efficiency, i.e. CD8⁺ cytotoxic T cells, natural killer (NK) cells, interferons and Abs. On the other hand, fine-tuning of the host defence strategies to avoid irreversible harm has led to activation of cellular and humoral immunity that tightly control herpesviral infections in a hierarchical but also redundant manner

* Corresponding author. Tel.: +49-1888-754-2502; fax: +49-1888-754-2328.

E-mail address: hengel@rki.de (H. Hengel).

[5]. To date, eight human herpesviruses, most of which are widespread in the population, are known (listed in Table 1). In the majority of cases, the viruses cause relatively mild or even asymptomatic infections in the immunocompetent host. However, when the intricate balance between viral immune escape and the host's immunity is lost, uncontrolled virus replication leads to severe disease manifestation as is frequently observed in immunocompromised patients or congenitally infected babies [6].

The complement system and antiviral Abs form a first line of antiviral defence operating before a virion is able to attach to its cellular receptor mediating entry into the host cell to initiate the replicative cycle [7]. The complement system includes proteolytic enzymes, inflammatory proteins, cell surface receptors and proteins that cause cell death through osmotic lysis after insertion into biological membranes. Therefore, a primary requirement is that the complement cascade becomes initiated only in response to infection and cannot be initiated in a random fashion. The complement factors opsonise and lyse Ab-coated viral particles (virolysis) and infected cells (cytolysis) [8,9]. A broad range of host cells expresses several complement-inhibiting proteins which protect tissues from complement-mediated lysis by the inhibition and dissociation of C3 convertases that are central for the amplification of the complement system [10]. Notably, the cell surface expression of CD46 and CD55, two of the complement control proteins (CCP), is strongly enhanced on the plasma membrane of HCMV-infected cells. The upregulated CD55 expression increases the capacity to regulate C3 deposition and thus protects CMV-infected cells from complement-mediated lysis

[11]. The selective incorporation of CCPs, i.e. CD55, CD59 and CD46, into virions and the discovery of virus-encoded regulators of complement activation (RCA) underscore the important antiviral role of the complement system [12,13].

Ab neutralisation of virus particles is achieved when bound Abs block the virion from productive interaction with receptors or prevent steps of entry and uncoating [14]. The combination of Abs and complement can activate the classical complement pathway to lyse enveloped viruses. The expression of the vFcyRs protects the HSV virion against this threat by interfering with the recruitment of complement [15].

The arsenal of immuno-modulatory mechanisms of HVs is not restricted to the protection of free virus particles. HV-infected cells are protected from destruction by cytotoxic effector cells and rendered refractory to antiviral cytokines like interferons to ensure efficient virus replication. Recent reviews delineate the bewildering multitude and sophisticated mechanisms of such defence strategies in great detail [3,4,16,17].

2. Antiviral effector functions of IgG

Ab neutralisation of viruses is usually demonstrated in vitro when binding of virus-specific Abs leads to a reduction in the number of infectious particles. The mechanisms leading to virus neutralisation are variable and include simple occupancy of virus surface and steric hindrance of a productive interaction with cellular receptors, causing virions to aggregate and thus reducing the concentration of infectious particles, but

Table 1
Human herpesviruses and their FcyRs

Subfamily	Designation	Synonyms	Genome size (kbp)	FcyRs	
				Genes	Proteins
α	HHV-1	Herpes simplex virus 1	152	<i>US8/US7</i>	gE/gI
	HHV-2	Herpes simplex virus 2	152	<i>US8/US7</i>	gE/gI
	HHV-3	Varicella-zoster virus	125	<i>ORF68/ORF67</i>	gE/gI
β	HHV-5	Cytomegalovirus	235	<i>UL119–118</i> <i>TRL11/IRL11</i>	gp68 gp34
	HHV-6	Human herpesvirus 6	162	–	–
	HHV-7	Human herpesvirus 7	162	–	–
γ	HHV-4	Epstein–Barr virus, Lymphocryptovirus	172	–	–
	HHV-8	Kaposi's sarcoma-associated herpesvirus	160	–	–

–: not reported.

also complex models involving capping of critical epitopes or induction of conformational changes in viral envelope or capsid structures [18,19]. The enhancing role of the Ab Fc part in direct neutralisation has been explained by an increased avidity of divalent Abs compared to monovalent Fab fragments [20], or its mere size in steric occupation of the virus surface [21].

Of paramount importance is the function of the Ab Fc moiety to recruit the complement system for enhancing *in vivo* neutralisation, by inducing Ab-dependent complement-mediated lysis (ADCL), and the Fc-receptor-mediated phagocytosis and subsequent destruction of the virus particle by professional antigen-presenting cells. The different ability of the IgG subclasses to bind complement can, therefore, explain the observation of differing neutralising capabilities of Abs sharing common variable regions but having different constant regions [22].

Only a minor fraction of the Ab repertoire that is generated during an immune response against a given virus has neutralising capacity. In addition, there are several other possible ways how Abs can act against viruses. Non-neutralising Abs may promote opsonisation and phagocytosis of a virus or contribute to antiviral defence during later stages of infection, when the virus has already entered the host cell. Recognition of viral antigens on the surface of infected cells by Abs can trigger virus-specific killing by nonspecific cytotoxic effector cells carrying Fc receptor (e.g. NK cells) in a process called Ab-dependent cellular cytotoxicity (ADCC) [23]. The Abs coating a virus-infected target cell attach to the effector cell via its Fc γ Rs, thereby stimulating the release of perforin and granzymes from cytolytic granula which destroy the virus-infected cell.

Moreover, Abs can also block the release of progeny virus from infected cells [24] and have been shown to prevent cell-to-cell spread of viruses [25]. Multimeric IgA and IgM molecules which are transcytosed through epithelial cells have intriguing functions even beyond those of IgG: when such Igs encounter viral antigens inside of cells, they can neutralise virus infectivity even at intracellular sites [26,27].

2.1. IgG in herpesviral infections

While Abs are powerful defence weapons against many viruses, their potency appears restricted against

HVs. HVs are commonly able to reactivate productive infection from latency and to reach horizontal transmission and spread in the presence of virus-specific Abs. Likewise, preexisting CMV-specific Abs frequently fail to prevent virus transmission or exogenous superinfection with new virus strains [28,29]. In mouse models of HV infection (mouse CMV, MCMV, murine γ -herpesvirus, MHV-68), Abs were demonstrated to be dispensable for the clearance of primary infection but only to become a limiting factor later on by preventing dissemination of recurrent virus during the post-primary phase of infection [30,31].

The therapeutic effectiveness of hyperimmune sera against HV is limited. It is of value for preventive rather than treatment purposes in certain clinical settings. Here we will discuss the clinical use of antiherpesviral IgG, concentrating on α - and β -HVs that express Fc γ Rs (see Section 4).

2.1.1. HCMV

In congenitally infected newborns, HCMV infection is an important cause of hearing loss as well as cognitive and motor impairments which cannot be effectively prevented or treated by current medical interventions (reviewed in Ref. [32]). Immunity to HCMV can strongly reduce the severity of disease, substantiating the need for a vaccine. In addition, HCMV infection is a major cause of disease and death in immunocompromised patients, e.g. solid organ and bone marrow transplant (BMT) recipients, cancer patients and HIV-infected individuals [33]. Pneumonia, retinitis and lesions of the gastrointestinal tract [34,35] are the most common manifestations of HCMV disease.

The applicability of highly efficient anticytomegaloviral agents like ganciclovir and foscarnet is often limited due to the toxicity of the drugs and the development of drug-resistant HCMV. Therefore, treatment with Abs is frequently a demanding option as additional or replacement therapy. The standard formulations are intravenous infusions of either immunoglobulins pooled from normal human plasma (intravenous Ig, IVIG), or from donors screened for high anti-CMV titers (CMV-IGIV). CMV-IGIV, distributed under the trade names CytoGam[®] and Cytotect[®], is indicated for prophylaxis of CMV disease associated with transplantation of kidney, lung, liver, pancreas and heart [36]. The latest therapeutic regimens include

the combination of antivirals like gancyclovir with CMV-IGIV [37,38], to increase the clinical benefit. The therapeutic value of CMV-IGIV has extensively been studied and reviewed [37,39–42]. Overall, most of the studies were able to demonstrate only a moderate if any reduction in CMV-associated morbidity in defined transplantation settings.

Due to the limited efficacy of polyclonal CMV-IGIV, IgG with a defined reactivity to HCMV antigens have been developed. The immunodominant CMV envelope glycoproteins gB and gH carry most of the neutralising B cell epitopes. They are involved in virus attachment, fusion with host cells and cell-to-cell spread of internal virions [43]. Therefore, gB and gH are obvious candidates for targeted Ab generation. The human monoclonal anti-gH-Ab MSL-109, or sevirumab[®], has neutralising capacity but failed to exert any beneficial effect in CMV-seropositive hematopoietic stem cell transplant (HSCT) recipients [44]. Similarly, other anti-HCMV Abs including humanised mouse monoclonal IgGs capable to neutralise HCMV in vitro have not proven effective for therapeutic application in vivo [45,46].

Several approaches using attenuated live virus or viral subunits, like gB, have been undertaken to produce HCMV vaccines, some of which were able to generate gB-specific Abs with demonstrable neutralising capacity [47]. However, no efficient HCMV vaccine is available for clinical purposes so far.

2.1.2. HSV

HSV-1 and HSV-2 share the ability to establish latent infection in sensory neurons and to accomplish recurrent cycles of reactivation, frequently leading to painful labial or genital lesions, respectively. Immunosuppression increases both the risk and the severity of HSV disease through cutaneous dissemination or infection of the central nervous system.

Several lines of circumstantial evidence suggest a protective effect of IgG against HSV-2. First, the risk of virus transmission and the extent of neonatal disease is much lower if the mother has recurrent as compared to primary genital infection [48]. Second, in mouse models of HSV-2 infection, various vaccination protocols generated Abs responses which mediated partial vaginal immunity [49,50]. Recent efforts for vaccine generation employed glycoprotein-D (gD) subunit formulations since gD is a target for neutral-

ising Abs due to the fact that gD directly interacts with HSV receptors on the cell surface to trigger virus entry. Two phase 3 studies of the GlaxoSmithKline HSV-2 gD-subunit vaccine demonstrated a 74% efficacy in preventing occurrence of genital herpes disease in women seronegative for both HSV-1 and HSV-2, while the vaccine was completely ineffective in decreasing HSV-2 infection in men or women seropositive for HSV-1 [51]. Similarly, the CHIRON gB2/gD2 vaccine using similar protein components, but administered with a different adjuvant, was ineffective in preventing HSV-2 infection [52].

2.1.3. VZV

VZV is the common etiological agent of two diseases, varicella (i.e. primary VZV infection or chickenpox) and zoster (i.e. shingles resulting from local reactivation of infection from endogenous VZV genomes in dorsal root ganglia cells). In immunocompetent children varicella is usually a benign, self-limited disease and no general treatment is required. In contrast, non-immune patients belonging to high-risk groups like immunocompromised individuals with exposure to VZV and newborns whose mothers undergo varicella at delivery must receive VZV immunoglobulins (VZIG). In these situations, varicella can take a life-threatening course of infection [53]. While VZIG is clearly efficient in post-exposure prophylaxis, it is not beneficial for therapy of already established VZV infection [54]. Herpes zoster occurs in the presence of VZV-specific Abs. Accordingly, VZIG has no preventive or therapeutic effect on herpes zoster, underlining the resilience of HVs to IgG.

In contrast to HCMV and HSV, active immunisation against VZV can be successfully accomplished with live attenuated virus and its use is approved in the United States since 1995. The VZV vaccine protects against severe varicella, although superinfection of vaccinees with exogenous wild-type VZV strains or reactivation of the vaccine virus may occur [55–57]. Early vaccination studies in children with leukemia suggest that the incidence of zoster might be lower in patients receiving the attenuated Oka vaccine strain than in those infected with wild-type virus [58], but at present it is not clear whether the vaccine will reduce the overall incidence of zoster in healthy vaccinated individuals.

Taken together, antiherpesviral IgG has a certain but limited prophylactic rather than therapeutic effectiveness in the control of HV spread and the progression and severity of disease. Abs alone, however, fail to provide sterile and protective immunity.

3. Cellular FcγRs

Specific surface-expressed cellular receptors are known for all Ig classes, including IgA (FcαR), IgD (FcδR), IgE (FcεR), IgG (FcγR) and IgM (FcμR). Further Ig receptors exert essential functions in distributing Abs, like the polymeric Ig receptor which transports IgA and IgM across epithelia [59] or the neonatal FcR (FcRn) which delivers maternal IgG to the foetus across the placenta [59]. Recently, a new family consisting of several members of B-cell-

expressed FcR homologs has been discovered [60], the precise function of which remains to be elucidated. All classes of cellular FcRs belong to the immunoglobulin supergene family (IgSF) of cell surface molecules. IgSF domains are characterised by a conserved structural motif termed the immunoglobulin fold (reviewed in Ref. [61]) that consists of two layers of antiparallel β-sheets, connected by variable loops. The Ig domain is an autonomous folding unit with manifold occurrence in various kinds of proteins.

The best studied and prototypic FcRs are the IgG-binding FcγRI, FcγRII, and FcγRIII which are exclusively expressed on immune cells and act at the interface of humoral and cellular immunity [62]. FcγRI (CD64) is a transmembrane protein with three extracellular Ig domains of the C2 class of Ig domains (see Fig. 1), which binds to human IgG-Fc with high affinity. The third, juxtamembrane domain

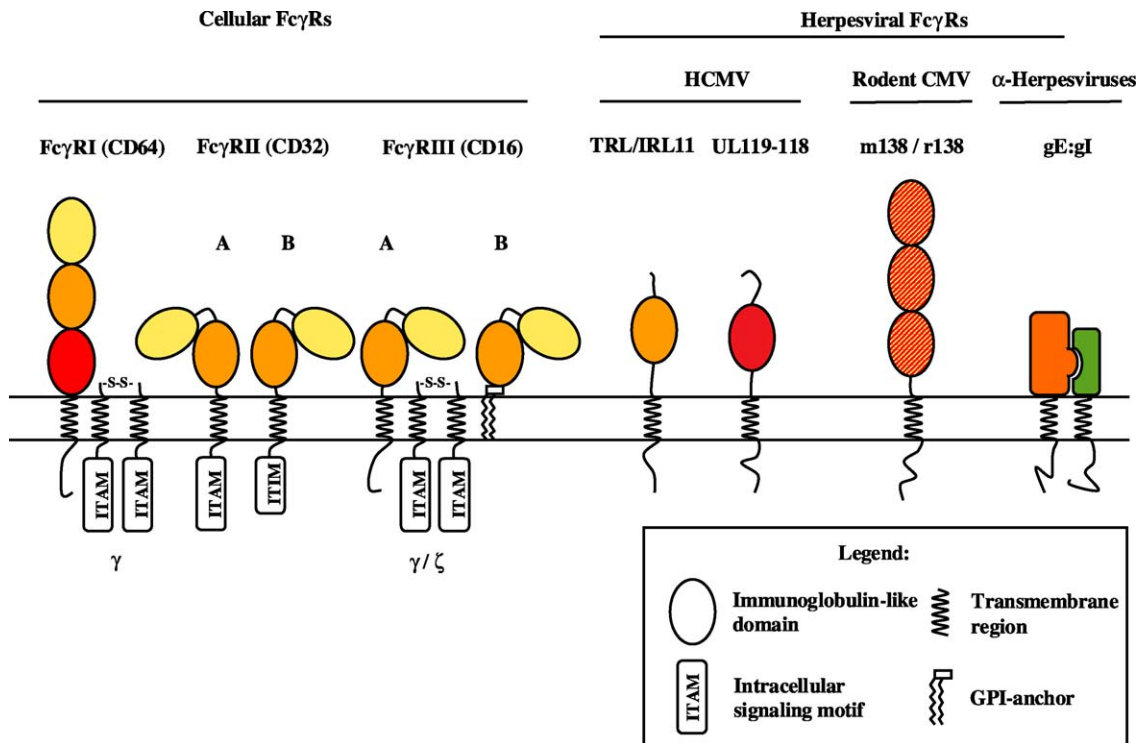


Fig. 1. Structural composition of cellular and herpesviral FcγRs. Predicted immunoglobulin supergene family (IgSF)-like domains of cellular (left) and herpesviral (right) FcγRs are colour-coded based on their sequence relatedness. The Ig-like domains of the rodent CMV FcγRs are similarly distant to each of the three domains of cFcγRI. The Ig domains of FcγRII and FcγRIII are depicted in their bent orientation that is seen in the crystal structures. The domain arrangement in the other FcγRs is unknown.

is responsible for the high affinity binding while the first two domains alone can mediate only weak binding to IgG [63]. Fc γ RI is the only Fc γ receptor which strongly binds to monomeric IgG molecules, but does not signal unless IgGs are cross-linked by their specific polymeric ligands.

Fc γ RII (CD32) and Fc γ RIII (CD16) preferentially bind polymeric, but not monomeric IgG. They possess two extracellular Ig domains with exhibit closest similarity to the membrane distal domains of Fc γ RI. The Fc γ RII isoforms A and B are transmembrane proteins differing in their cytoplasmic portion, while Fc γ RIII can be expressed as a transmembrane or GPI-linked form (Fig. 1) [64]. The cellular Fc γ Rs use distinct intracytoplasmic motifs for signal transduction into the cell. Fc γ RIIA and Fc γ RIIB contain an “immunoreceptor-tyrosine-based activation motif” (ITAM) or “immunoreceptor-tyrosine-based inhibitory motif” (ITIM), respectively, in their cytoplasmic tail. Both motifs are phosphorylated upon receptor engagement but with opposed outcome: While the ITAMs preferentially recruit src-family tyrosine kinases and induce cellular activation, ITIM phosphorylation serves to terminate signaling, mostly via the SH2-domain-containing inositol phosphatase (SHIP). Fc γ RI and Fc γ RIII do not contain intrinsic signaling properties but share the common FcR γ chain. Alternatively, Fc γ RIII can utilise the closely related ζ chain of the T-cell-receptor complex. The FcR γ and the ζ chain each contain an ITAM motif to induce activatory signaling events like the Fc γ RIIA cytoplasmic moiety [62]. The cross-linking of Fc γ RI-bound IgG by multivalent antigens, or the binding of preformed immune complexes with Fc γ RII or Fc γ RIII, results in clustering of the Fc γ R and triggering of a variety of effector mechanisms, such as ADCC, phagocytosis, the release of cytokines, enhanced antigen presentation, and the regulation of Ig production by B cells [62]. Thus, cFc γ Rs can be functionally considered as antigen receptors.

The Fc portion of Ig forms a well-characterised structure [65]. Its binding to cFcRs is mediated by the membrane-proximal second IgSF domain of cFcRs as revealed by recent studies resolving the crystal structures of Fc γ RIII in complex with IgG-Fc [66], Fc γ RIIA complexed with IgG-Fc [67] and Fc ϵ RI bound to IgE-Fc [68]. These studies revealed a con-

served arrangement of the two Ig domains in a highly bent conformation, uncovering the Ig binding site that is located at the top of the second Ig domain concordantly in all three molecules.

Interestingly, all three Fc γ Rs also occur as soluble forms in the serum, generated either by proteolysis [69], alternative splicing [70], or from separate genes [63]. The functional significance of these isoforms remains to be unravelled.

Studies taking advantage from gene knock-out mice have demonstrated prominent immunoregulatory properties of cFc γ Rs. Mice devoid of the common FcR γ chain display pleiotropic defects of immune effector cells including impaired macrophage phagocytosis and NK-cell-mediated ADCC as well as defective mast cell-dependent allergic responses [71]. The similar phenotype of Fc γ RIII-deficient mice illustrates the intimate functional cooperation with the γ chain [72]. The critical regulatory (inhibitory) effect of Fc γ RIIB on various immune cells is reflected by the hyperreactivity of the immune system in Fc γ RIIB-deficient animals. Due to the lack of control of B cell proliferation, Fc γ RIIB-deficient mice develop various forms of autoimmune diseases, either spontaneously or after experimental induction [73,74]. Mast cells from Fc γ RIIB-deficient animals are hypersensitive to IgG-induced degranulation which culminates in enhanced passive cutaneous anaphylaxis reactions [74].

4. Structure and functions of herpesviral Fc γ Rs

While the cellular Fc γ Rs play a key role in transducing the antiviral potency and specificity of IgG to cellular immunity, various pathogens exploit the same principle of binding the IgG Fc proportion for their own purpose to avoid immune destruction. Examples for Fc-binding proteins are found in gram-positive bacteria [75], in protozoa like schistosomes and trypanosomes [76–78], in coronaviruses [79], hepatitis C virus [80], and most commonly in α - and β -herpesviruses. The last group comprises cytomegaloviruses from different species [81–83], herpes simplex viruses 1 and 2 [84], varicella zoster virus (VZV) [85], the porcine herpesvirus pseudorabies virus (PRV) [86] and presumably also Marek’s disease virus (MDV), an α -herpesvirus infecting chickens,

where proteins homologous to the α -herpesvirus Fc γ Rs are found [87].

4.1. The Fc γ R of the α -herpesviruses: the gE:gI complex

The ability of HSV-1-infected cells to bind human IgG via its Fc-part was first demonstrated in 1979 [88]. The responsible membrane proteins have subsequently been identified as a complex formed by the glycoproteins gE and gI (Fig. 1) [89], which are encoded by the HSV genes *US* (unique short) 7/*US8* and are dispensable for HSV infection and replication in vitro. Homologous molecules to gE and gI are found in HSV-2 [84], VZV [90], PRV [91] and MDV [87]. gE and gI are exposed on the surface of infected cells and represent also the most abundant structural glycoproteins within the virus envelope. Alignment of a limited portion of the extracellular chain of gE reveals a low sequence similarity with the second domain of cFc γ Rs [92]. While gE alone binds only aggregated IgG with low affinity, complex formation with gI induces a high affinity receptor which is able to fix monomeric IgG. The HSV-1 gE:gI complex is a heterodimer composed of one molecule each and binds IgG with 1:1 stoichiometry. The affinity for IgG₄ is highest with $K_D=40$ nM [93]. The gE:gI complex binds IgG between the second and third Ig domain, at the C γ 2–C γ 3 interface, which is similar to the Fc-binding site of rheumatoid factors and protein A from *Staphylococcus aureus*. The importance of H435 of IgG1 for the interaction correlates with the failure to bind many IgG3 subclass allotypes, where the corresponding residue is an arginine [93].

The IgG-binding capability of gE:gI protects HSV-infected cells from ADCC [15]. In mice passively immunised with human anti-HSV-IgG, titers of a gE mutant with a 4-aa insert disrupting the region homologous to mammalian Fc γ Rs were significantly reduced, whereas wild-type HSV remained unaffected. [94]. The minimal mutation introduced into the gE mutants affected the IgG-related functions of gE:gI, but did not interfere with the other important gE function, i.e. to mediate direct cell-to-cell spread of nascent virions [95].

The observed protection against antiviral IgG conferred by gE:gI was explained by “antibody bipolar bridging” (ABB) [96] (Fig. 2). According to this

model, an IgG molecule recognising an epitope on the surface of a virus-infected cell or a virion via its clonotypic antigen binding Fab domain is simultaneously sequestered by the gE:gI complex via its Fc part. Thus, the IgG Fc domain is prevented from recruiting effector mechanisms, i.e. the complement component C1q or Fc γ R-expressing cytotoxic effector cells. Several lines of evidence support this hypothesis. First, the competition of binding sites between gE:gI and protein A on the Fc–C γ 2–C γ 3 interface was exploited to demonstrate the masking of the Fc part of IgG through the viral Fc γ R when bound to HSV-infected cells via its antigen-binding sites [97]. Second, fluorescence resonance energy transfer (FRET) studies have demonstrated that the IgG molecule structure is indeed equipped with sufficient intrinsic flexibility allowing the simultaneous interaction of the Fab and Fc domains with separate ligands displayed on the same cell or virion surface [98].

The VZV gE vFc γ R was demonstrated to undergo repeated cycles of receptor-mediated endocytosis and recycling to the plasma membrane of VZV-infected cells. This function depends on a tyrosine residue in a conserved YXXL motif that resembles endocytosis signals [99]. Most interestingly, this process is induced by Fc binding to gE. This finding suggests that the vFc γ Rs might be designed as a molecular device removing antiviral IgG from the surface of infected cells and thus avoids ADCC and ADCL.

4.2. HCMV Fc γ Rs

Biochemical evidence for an HCMV-induced Fc-binding activity in infected fibroblasts has been collected over almost three decades. IgG binding was detected on the plasma membrane and at intracellular sites of infected cells, as well as in association with HCMV virions [100]. Fc binding was ascribed to various proteins with MWs between 38 and 130 kDa [101]. Only recently, three viral genes encoding two different Fc binding glycoproteins, gp68 and gp34, were identified within the HCMV AD169 genome, both of which are dispensable for viral replication in vitro [83,102]. The equipment with two distinct Fc γ Rs is unique for HCMV and adds another example to the bewildering array of HCMV glycoproteins by which this HV modulates immune functions. The structural composition of the HCMV-encoded vFc γ Rs

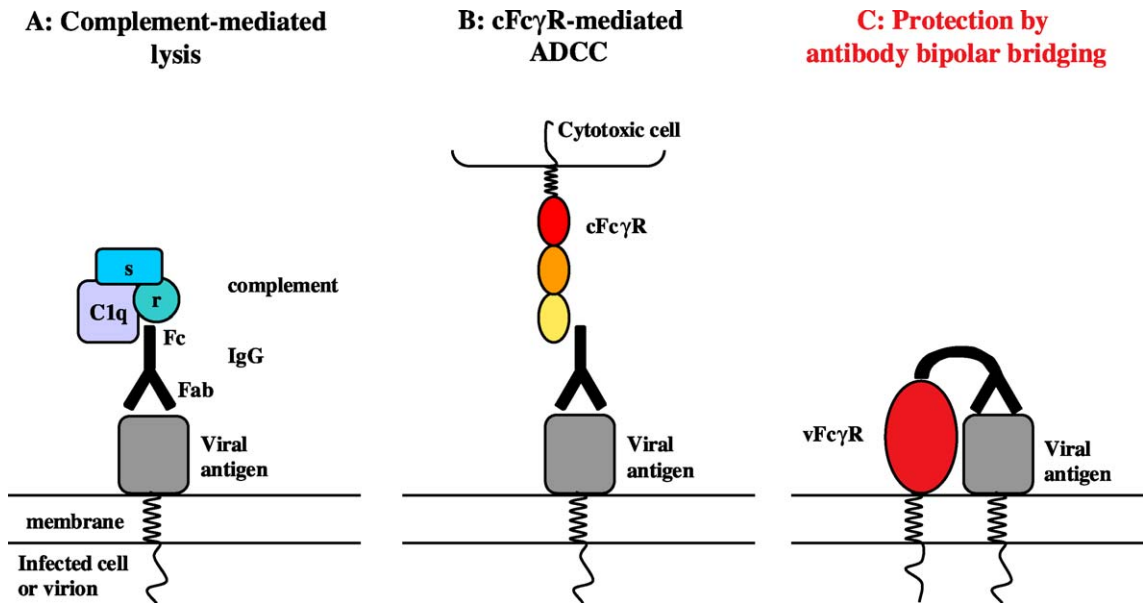


Fig. 2. Herpesviral FcγRs protect against antiviral IgG by antibody-bipolar bridging. (A) Antiviral IgG binds to its cognate viral antigen on the surface of an infected cell or a virus particle via its Fab part. Upon binding, the Fc region recruits the C1q,r,s complex which activates the complement system via the classical pathway, resulting in antibody-dependent complement-mediated lysis (ADCL). (B) Alternatively, attachment of bound virus-specific IgG to cFcγR⁺-cytotoxic effector cells, e.g. NK cells, mediates cFcγR cross-linking and triggering of antibody-dependent cellular cytotoxicity (ADCC). (C) Antibody-bipolar bridging: viral FcγRs sequester the Fc part of bound antiviral IgG. This engagement blocks the recruitment of complement proteins or cFcγRs and thus mediates protection of the virus particle or infected cell from destruction.

is thoroughly different from the HSV gE:gI heterodimer in that the former represent single-chain type I transmembrane proteins showing significant sequence homology with human cFcγRs in their extraluminal part and are predicted to form IgSF-like domains [83] (Fig. 1).

gp68 is expressed during the early and late phase of HCMV infection from a hitherto unidentified spliced 4.1 kb transcript encompassing the open reading frames *UL119* and *UL118* from a complex transcription unit, *UL115–119*. The *UL119–118* gene sequence encodes a 347-aa type Ia transmembrane protein with 12 potential *N*-glycosylation sites. The extracellular domain is predicted to contain a single V-like Immunoglobulin superfamily (IgSF) domain (aa 90–190), including a conserved disulphide bond. In comparison to cellular FcγRs, this domain displays the highest degree of homology to the unique third IgSF domain of FcγRI, the domain conferring the high affinity binding to IgG. Secondary structure algorithms concordantly predict a composition of the

gp68 IgSF domain built by seven β-sheets resembling the architecture of the cFcγR IgSF domains [83]. The cytoplasmic domain of gp68 contains a modified ITIM motif (WSYKRL) which may provide a link of gp68 to cellular signaling pathways.

The glycoprotein gp34 is encoded by two identical gene copies, *TRL11* and *IRL11*, the latter of which is present in the duplicated ULS' (unique long) segment of the laboratory HCMV strain AD169. gp34 is a type Ia transmembrane protein consisting of 234 aa. The extracellular region includes three potential *N*-glycosylation sites and is predicted to form one IgSF-like domain [83]. This prediction is based on homology with the second domain of the cFcγRIII/RII, which forms the essential IgG binding moiety of cFcγRs [103]. Important determinants of the three cFcγRIII binding regions for Fc fragment are conserved in the gp34 sequence. Interestingly, secondary structure calculations predict α-helical interruptions of the β-sheet structure of the IgSF domain, a feature which resembles the FcRn, where α-helices also make important

contributions to the binding interface with hIgG [104]. The cytoplasmic tail of gp34 contains a conserved dileucine motif (DXXXLL) which could indicate a role in intracellular targeting of IgG–gp34 complexes to the endocytic route [105]. Thus, similar to VZV gE:gI, HCMV gp34 might remove bound IgG from the plasma membrane of infected cells to prevent ADCL and ADCC. In contrast to the HSV gE:gI FcγR, HCMV-infected cells were shown to bind to all 4 subclasses of human IgG [106] with graduated affinity (IgG1 ≥ IgG4 > IgG2 > IgG3 [107]). However, the experimental setting used could not dissolve the separate Ig binding properties of gp34 and gp68. These are likely to exist given the different affinities to IgG of distinct mammalian species. Among non-human IgG, rabbit and, to a minor extent, rat IgG is recognised by gp34 while no binding was observed for gp68 [83,106].

Ultrastructural information revealing the exact binding and contact sites of the HCMV FcγRs and the Fc ligand is not available yet. However, one conclusion with regard to the Fc molecule can be drawn from the fact that Fc-bound vFcγRs can still be precipitated with protein A [83] implying that separate binding sites must exist. This feature of HCMV FcγRs is contrary to HSV gE:gI where the *S. aureus* protein A competes for Fc binding.

Similar to the HSV gE:gI, both of the HCMV FcγRs are independently interfering with the neutralising function of human IgG (Reinhard, H. and Hengel H., unpublished observation). This fact is also evident from plaque reduction assays with HCMV-immune human sera performed in the presence of an excess of Fc fragments or β₂-microglobulin (β₂m) as a control protein. While the addition of Fc but not β₂m improves the neutralising efficiency of HCMV-IgG in a dose-dependent manner, the activity of Measles virus (MV)-specific IgG is completely independent of Fc when neutralising MV virions which do not possess vFcγRs (Reinhard, H. and Hengel H., unpublished observations). This finding may have obvious implications for the in-vivo neutralisation of HCMV: In body fluids like blood, the high amount of non-immune IgG might exert a similar effect like the Fc fragment in the neutralisation assay discussed above, i.e. enhance HCMV virion neutralisation. In contrast, at sites where IgG concentrations are significantly lower than in serum, i.e. on mucosal surfaces and body fluids like saliva, breast milk and genital fluids the HCMV vFcγRs should

promote virus shedding and horizontal transmission. In such an environment vFcγRs are critically required to confer resistance of HCMV particles against neutralising IgG.

Notably, the HCMV vFcγRs could also provide a molecular link for infection and replication of the human immunodeficiency virus (HIV). The HIV-induced damage of the host's immune system favours HCMV reactivation from latency and the emergence of persistent HCMV replication and recurrent disease [108]. In this context, it is interesting that HCMV infection is able to confer HIV-susceptibility to otherwise nonpermissive fibroblasts [109]. This could be explained by Ab-coated HIV particles attaching via the vFcγRs to the surface of HCMV-infected cells. By this mechanism HIV virions might be endocytosed and establish HIV infection. This could open up a cellular compartment for HIV replication where HIV may benefit from the multitude of stealth mechanisms by which HCMV avoids immune attack.

4.3. Rodent CMV FcγRs

Mouse CMV (MCMV)-infected cells can be decorated with IgG-Fc fragment both intracellularly and on the surface [82]. The genetic basis for this feature is the expression of a single gene, *m138*, coding for an FcγR [82]. The predicted 569-aa type I transmembrane glycoprotein has a calculated mass of 65 kDa and is synthesised during the early and late phase of MCMV replication. The MCMV FcγR is highly glycosylated reaching a MW of 105 kDa, which is reduced to a single band of 68 kDa by deglycosylation. The *m138* protein can be precipitated with mouse IgG-Fc fragment coupled to protein A indicating non-overlapping binding sites as it is the case for the HCMV FcγRs. Alignment of the *m138* sequence with mouse cFcγRs displays a more significant degree of homology and predicts a composition of three FcγR-related IgSF-like domains. (Fig. 1, A. Zimmermann, A. Bigl and H. Hengel, unpublished data).

Two distinct and independent *m138* homologs are present in two different cytomegaloviruses of the rat (RCMV), RCMV strain Maastricht, and RCMV strain England, respectively [110–112]. Preliminary data show that both RCMV-encoded *m138* homologs constitute functional vFcγRs (A. Bigl, M. Budt, S. Voigt, A. Zimmermann, unpublished observation). The

MCMV model provides an unique opportunity to assess the biological role of an vFcγR for viral pathogenesis and immune control in vivo. To this end the replication of a MCMV *m138* gene deletion mutant, $\Delta m138$, was analysed in mice and revealed a dramatically attenuated phenotype in vivo. Surprisingly, in $\mu\text{mt}^{-/-}$ mice lacking the Ig μ chain, defective in B cell development and devoid of Abs, the $\Delta m138$ virus was not rescued from attenuation. From this finding it was concluded that the major biological function of the MCMV FcγR must not be related to IgG binding but includes a more prominent IgG-unrelated function as it is known for HSV gE:gI [113]. However, the replacement of the *m138* gene sequence by inserting the *LacZ* gene of *Escherichia coli* might have affected genes neighbouring *m138*, which possess common transcription units with *m138* [82] and have been implicated in macrophage tropism and efficient MCMV replication in vivo [114]. Furthermore, the viral replication was investigated during primary MCMV infection, where Abs do not contribute to virus immune control [31]. Therefore, the available data do not preclude an Ab-dependent role for the MCMV FcγR at later phases of infection when Abs effectively limit virus dissemination and transmission [5,31]. Studies introducing more subtle changes in the *m138* gene without affecting adjacent genes are required to elucidate putative IgG-dependent functions of the MCMV FcγR.

5. Perspective: engineering virus-specific IgGs resisting vFcγRs?

Recent progress in Ab generation technologies allows to select Abs of perfect specificity for defined target structures which can employ distinct effector functions and can be produced in high quantities allowing therapeutic application in patients [115]. Due to the limitations of conventional antiviral drug therapy, the design of potent virus-specific Abs as a therapeutical tool is demanding. Published data support the notion that IgG has an important but certainly not exclusive immunological role against herpesviral infections, and is a potential tool of immunoprophylaxis [19,116]. As outlined above, viral FcγRs are prime suspects for the remarkable ability of HVs to counteract IgG effector functions. Therefore, it is conceivable to assume that antiviral Abs could reach

a more potent antiviral efficiency when resisting FcγR-mediated attenuation. According to this concept, an ideal IgG-Fc domain would not be bound by a vFcγR, while still retaining normal affinity to the ligands that recruit protective components of the immune system. As an example, human IgG₃ Abs could represent promising candidates for an effective anti-HSV agent since this IgG subclass is a potent activator of the complement cascade [117], but is not blocked by HSV gE:gI [93]. Based on the discrimination of the HSV gE:gI FcγR between hIgG₁ allotypes [118], even minimal mutations introduced into recombinant Abs could be sufficient to avoid vFcγR binding, while retaining the functional properties of the molecule with respect to cFcγRs and complement.

HCMV encodes even two FcγRs which bind all human IgG subclasses [83], although with graded affinity, making the design of a discriminating IgG-Fc domain more complex. A detailed understanding of the interaction of the CMV FcγRs with IgG-Fc including ultrastructural analysis would facilitate the modelling of effective IgGs against this virus. If a prototypic IgG-Fc domain fulfilling the requirements of selective interaction is found, this module could be fused to a large number of HCMV-reactive IgG-Fab domains. Such a cocktail of monoclonal recombinant Abs will be necessary to deal with the interstrain diversity of the major HCMV neutralising epitopes present on the glycoproteins gB and gH [119–121] in order to generate widely effective protection.

The MCMV animal model could serve as an optimal test tube to generate experimental evidence in vivo for an IgG-Fc-dependent subversive role of vFcγRs in viral pathogenesis in a natural host. Next, as a proof of principle, MCMV-specific monoclonal Abs with a therapeutic potential [116] are available which will allow to assess the therapeutic benefit of a mutated vFcγR-resistant IgG-Fc module in a variety of infection conditions in vivo. Based on this knowledge, designed IgGs could lead to a new generation of immunopharmacological tools effective against the large burden of human herpesviral diseases.

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